REMARKS

The Official Action dated November 8, 2000, and the Advisory Action dated February 20, 2001, have been carefully considered. Accordingly, the changes presented herewith, taken with the following remarks, are believed sufficient to place the present Application in condition for allowance. Reconsideration is respectfully requested.

Claims 8-9, 11-20, and 32-33 have been canceled. Claims 1 and 22-23 have been amended. Support for the amendment to Claim 1 can be found in the specification as originally filed on page 4, lines 10-11 and page 5, lines 1-3. Claims 22-23 have been amended as to form.

The same claim amendments were submitted in the Amendment filed February 5, 2001. In the Advisory Action the Examiner indicated that the amendments would be entered upon filing of a Notice of Appeal and Appeal Brief. The Notice of Appeal was filed March 2, 2001. In order to assure consideration of the Declaration submitted herewith, rather than filing an Appeal Brief the case has been refiled. The claims are supported by the specification and add no new matter, whereby entry is in order.

The present invention relates to an isolated nucleic acid comprising a sequence selected from:

(a) a sequence according to nucleotide No. 372 to nucleotide No. 2681 of SEQ ID NO: 1, nucleotide No. 335 to nucleotide No. 1822 of SEQ ID NO: 3 and nucleotide No. 95 to nucleotide No. 1597 of SEQ ID NO: 5,

(b) a sequence hybridizing with any of the sequences defined under (a) in 2 x SSC at 60°C,

(c) a sequence complementary to the sequences defined under (a), and

(d) a sequence which, due to degeneracy of the genetic code, encodes the same amino acid sequences as those encoded by the sequences defined under (a),

wherein the nucleic acid encodes a complete or partial insect acetylcholine receptor subunit having the ability to form homooligomeric acetylcholine receptors when expressed in host cells, as recited in Claim 1.

The invention also relates to vectors and host cells comprising the nucleic acid. According to Claim 10, the invention further related to a process for preparing a polypeptide encoded by a nucleic acid of Claim 1 comprising:

(a) culturing a host cell containing a nucleic acid of Claim 1 or a vector comprising at least one nucleic acid of Claim 1 under conditions which ensure expression of the nucleic acid of Claim 1, and

(b) isolating the polypeptide from the cell or the culture medium.

Claims 1-7, 10, 17 and 22-31 have been rejected under 35 U.S.C. § 112, first paragraph, as not being supported by an enabling specification. The Examiner alleges that the specification does not reasonably provide enablement for any and all partial sequences which are at least 14 base pairs in length of the sequences according to SEQ ID NOS: 1, 3 or 5, or any and all sequences which exhibit at least 70% identity between position 1295 and position 2195 from SEQ ID NO: 1, or between position 432 and position 1318 from SEQ ID NO: 3, or between position 154 and position 1123 from SEQ ID NO: 5. The Examiner further alleges that the specification fails to teach which of the claimed nucleic acids encode a polypeptide which can be used as intended, i.e. to screen for pesticides, and that the Applicants have failed to show how to determine which of the claimed nucleic acids can be used as intended.

Claim 1, as amended, no longer recites a partial sequence at least 14 base pairs in length of the sequence defined under (a) or a sequence exhibiting at least 70% identity with any of the sequences defined under (a) between position 1295 and position 2195 from SEQ ID NO: 1, between position 432 and position 1318 from SEQ ID NO: 3, or between position 154 and position 1123 from SEQ ID NO: 5. As will be set forth below, Applicants submit that Claim 1, and Claims 2-7, 10 and 22-31 dependent directly or indirectly, thereon are supported by an enabling specification. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

In the Advisory Action the Examiner alleges that a nucleic acid of present Claim 1 encodes a polypeptide which can form part of a functional acetylcholine receptor (AChR) in the presence of an alpha subunit, but that there is no evidence that the claimed nucleic acids encode a functional homooligomeric receptor. It appears that the Examiner is alleging that the alpha subunit of the L-type voltage-gated Ca^{2+} channel and the polypeptide encoded by the claimed nucleic acid

together form the AChR. However, the polypeptide encoded by the claimed nucleic acid forms a homooligomeric AChR, and the alpha subunit of the L-type voltage-gated Ca^{2+} channel is not part of the AchR.

Nicotinic acetylcholine receptors are oligomeric (Breer and Sattelle, *J. Insect Physiol.*, 33, 771-790 (1987); Elliot et al., WO 96/41876; Stetzer et al., *FEBS Letters*, 398, 39-44 (1996); Schulz et al., *J. of Neurochem.*, 7, 854-862 (1998)). Breer and Sattelle, *J. Insect Physiol.*, 33, 771-790 (1987) teach that the binding of acetylcholine to nicotinic acetylcholine receptors (nAChRs) triggers an increase in conductance to the cations Na^+, K^+ and Ca^{2+} via a transmembrane aqueous channel, resulting in depolarization of the cell membrane.

Elliot et al., WO 96/41876, teach that activation of nAChRs leads to an influx of cations, including Ca^{2+}. Elliot et al. further teach that the Ca^{2+} entry into the cell can induce release of calcium contained in intracellular stores, while monovalent cation entry into the cell can result in an increase in cytoplasmic Ca^{2+} levels through depolarization of the cell membrane and subsequent activation of voltage-dependent calcium channels. Thus, the voltage-dependent calcium channels are not part of the nAChRs, and an increase in intracellular calcium concentrations can be utilized to analyze nAChR expression.

Elliot et al. also teach the use of calcium-sensitive indicators wherein the interaction of the indicator with calcium results in increased fluorescense of the indicator, therefore, an increase in intracellular Ca^{2+} concentration of cell containing the indicator can be expressed as an increase in fluorescence. Elliot et al. disclose that HEK-293 cells which were transfected with DNA encoding a human neuronal nAChR exhibit increased fluorescence after exposure to nicotine, and that HEK-293 cells which were untransfected did not exhibit a change in fluorescence after exposure to nicotine.

Stetzer et al., *FEBS Letters,* 398, 39-44 (1996), teach human embryonic kidney HEK-293 cells which stably express an L-type voltage-gated Ca^{2+} channel can be genetically modified to stably express a rat neuronal nicotinic acetylcholine receptor (nAChR), and that the Ca^{2+} channels are activated by nAChR-mediated depolarization of the cell membrane. Stetzer et al. further teach Ca2+ imaging with the Ca^{2+}-chelating agent fura-2 as an assay permitting sensitive detection of nAChR-expressing cell clones.

Thus, one of ordinary skill in the art would, from reading the references, appreciate that the alpha subunit of the L-type voltage-gated Ca^{2+} channel is not a part of the AchR.

Further, as shown in the data set forth in the Declaration of Dr. Martin Adamczewski, HEK-293 cells transfected with nucleic acids in accordance with the invention exhibit increase cellular calcium levels in response to nicotine, thus the polypeptides encoded by the claimed nucleic acids form functional nicotinic acetylcholine receptors. Importantly, the data set forth in the specification and the Declaration of Dr. Adamczewski were obtained with cells transfected with only one claimed nucleic acid, thus, the nAChRs formed are homooligomeric and not heterooligomeric.

For example, the data set forth in the specification were obtained using HEK-293 cells transfected with a construct comprising the Hva7-1/5HT3 chimera described on page 14 of the specification. The chimera comprises a nucleic acid in accordance with the invention. The cells were not transfected with any other inventive nucleic acid. Thus, the receptor was formed from the polypeptide encoded by the Hva7-1/5HT3 chimera. As the oligomeric receptor was formed from a single type of polypeptide, the receptor was homooligomeric. As indicated by Figure 1, the cells responded to nicotine by exhibiting an increased calcium level, thus the receptor was functional.

The Declaration of Dr. Adamczewski sets forth additional data generated using HEK-293 cells transfected with constructs comprising the Da7 clone (Figure 2), the Hva7-1 clone (Figure 3) or the Hva7-2 clone (Figure 4), described on pages 13-14 of the specification, all of which are nucleic acids in accordance with the invention. In each experiment the cells were transfected with a single clone, thus the resulting receptors were formed from a single type of polypeptide and are homooligomeric. As indicated by Figures 2-3, in each experiment the cells responded to nicotine by exhibiting an increased calcium level, thus the receptor was functional.

Additionally, the specification teaches that acetylcholine receptors are synthesized from polypeptides which are encoded by the nucleic acids according to invention (page 5, lines 1-4) and that the acetylcholine receptors are homooligomeric (page 4, line 11).

Nucleic acids which encode a complete or partial insect acetylcholine receptor subunit having the ability to form homooligomeric acetylcholine receptors when expressed in host cells, as recited in Claim 1, encode a polypeptide which can be used to screen for insecticidal substances. The specification teaches that the nucleic acids according to the invention may be used to discover insecticidal substances by introducing a recombinant DNA molecule encompassing at least one nucleic acid in accordance with the invention into a host cell, culturing the host cell under conditions which permit expression of the receptors and in the presence of a compound to be tested, and detecting any change in receptor properties (page 6, line 28-page 7, line 6).

A suitable culturing method and method for detecting changes in receptor properties are set forth on page 14, line 25-page 15, line 26. Briefly, the host cells may be cultured in Dulbecco's modified Eagle's medium supplemented with fetal calf serum and Zeocin. As acetylcholine receptors are ligand-regulated ion channels, changes in receptor properties may be determined by detecting alterations in intracellular calcium concentration by treating the host cells in the presence of the compound to be tested with Fura-2-acetoxy methyl ester, and determining fluorescence intensity before and after treatment with a ligand (nicotine).

Thus, the specification teaches one of ordinary skill how to determine whether a polynucleotide encodes an acetylcholine subunit having the ability to form acetylcholine receptors, as required by Claim 1. Further, the specification teaches one of ordinary skill how to determine whether the nucleic acids can be used as intended, namely, how to use the nucleic acids to determine whether compounds modulate the conducting properties of acetylcholine receptors. A disclosure is enabling if one reasonably skilled in the art could make or use the invention from the disclosure, coupled with information known in the art, without undue experimentation. *United States v. Telectronics, Inc.*, 857 F.2d 778, 785 (Fed. Cir. 1988) (citation omitted), cert. denied, 490 U.S. 1046 (1989). That some experimentation is necessary does not preclude enablement. *Atlas Powder Company v. E.I. Du Pont De Nemours & Company*, 224 USPQ 409, 413 (Fed. Cir. 1984) (citations omitted)

The specification further teaches that nucleic acids according to the invention may be used to discover compounds which bind to receptors, and that these

compounds can be used as plant insecticides (page 7, lines 8-14). Host cells containing a nucleic acid according to the invention and expressing the acetylcholine receptors synthesized from polypeptides which are encoded by the nucleic acids are brought into contact with the compound to be tested and interactions between the compound and the host cells, receptors and/or polypeptides can be evaluated.

Therefore, for the reasons set forth above, Claim 1, and Claims 2-7, 10 and 22-31 dependent directly or indirectly, are supported by an enabling specification, whereby the rejection under 35 U.S.C. § 112, first paragraph, should be withdrawn.

Claims 1-7, 10, 17 and 22-31 have been rejected under 35 U.S.C. § 112, second paragraph, as indefinite. The Examiner alleges that Claims 1-7, 10, 17 and 22-31 are indefinite as it is unclear what is intended by "partial acetylcholine receptor". The Examiner alleges that Claim 17 is further indefinite because it recites "[t]he regulatory sequence of claim 3" and "the nucleic acid" without proper antecedent basis, and because it is unclear which regulatory sequence and which amino acid is intended.

As will be set forth below, Applicants submit that Claim 1, and Claims 2-7, 10 and 22-31 dependent directly or indirectly thereon, are definite. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

Claim 1 recites an isolated nucleic acid which encodes a complete or partial insect acetylcholine receptor subunit having the ability to form homooligomeric acetylcholine receptors when expressed in host cells. Applicants submit that one of ordinary skill will appreciate that a "partial acetylcholine receptor subunit having the ability to form homooligomeric acetylcholine receptors when expressed in host cells" refers to a protein comprising fewer amino acids than the complete native protein but which still exhibits an ability to form homooligomeric receptors. Whether a claim is indefinite requires an analysis of "whether one skilled in the art would understand the bounds of the claim when read in light of the specification. If the claims read in light of the specification reasonably apprise those skilled in the art of the scope of the invention, 112 demands no more." *Miles Lab., Inc. v. Shandon Inc.*, 997 F.2d 870, 875, 27 USPQ2d 1123, 1126 (Fed. Cir. 1993), cert. denied, 114 S. Ct. 943 (1994).

Claim 17 has been canceled, whereby the rejection as to Claim 17 has been overcome.

For the reasons set forth above, Claims 1-7, 10 and 22-31 are definite, whereby the rejection under 35 U.S.C. § 112, second paragraph, should be withdrawn.

Claim 1 has been rejected under 35 U.S.C. § 102 as being anticipated by Schulte et al. The Examiner alleges that Schulte et al. teach GenBank Accession No. AF143486, which is 97.8% identical to SEQ ID NO: 1 and 100% identical to SEQ ID NO: 3 between positions 432 and 1318, and GenBank Accession No. AF143487, which is 97.34% identical to SEQ ID NO: 5 and 100% identical to SEQ ID NO: 5 between positions 154 and 1123.

As will be set forth below, Applicants submit Schulte et al. is not a proper reference. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

A certified translation of the priority document, German Application 19819829.9, was filed October 27, 2000, whereby Applicants have perfected a claim for the priority date of May 4, 1998. Thus Schulte et al., published by deposition in GenBank on April 19, 1999, is not a proper § 102 reference, whereby the rejection of Claim 1 under 35 U.S.C. § 102 based on Schulte et al. is overcome.

Claim 1 has been rejected under 35 U.S.C. § 102 as being anticipated by Celniker et al., Liao et al., or Vogel. The Examiner alleges that Celniker et al. teach GenBank Accession No. AC004326, which is 99.4% identical to bases 9-836 of SEQ ID NO: 1 and which contains 340 bases identical to bases 496 to 836 of SEQ ID NO: 1. Further, the Examiner alleges that Liao et al. teach GenBank Accession No. AF045432 which is identical to SEQ ID NO: 3 over a length of 79 bases, and that Vogel et al. teach GenBank Accession No. Z97178 which is identical to SEQ ID NO: 5 over a length of 98 bases.

As will be set forth below, Applicants submit that Claim 1 is not anticipated by Celniker et al., Liao et al., or Vogel et al. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

As discussed above, Claim 1 recites an isolated nucleic acid comprising a sequence selected from:

(a) a sequence according to nucleotide No. 372 to nucleotide No. 2681 of SEQ ID NO: 1, nucleotide No. 335 to nucleotide No. 1822 of SEQ ID NO: 3 and nucleotide No. 95 to nucleotide No. 1597 of SEQ ID NO: 5,

(b) a sequence hybridizing with any of the sequences defined under (a) in 2 x SSC at 60°C,

(c) a sequence complementary to the sequences defined under (a), and

(d) a sequence which, due to degeneracy of the genetic code, encodes the same amino acid sequences as those encoded by the sequences defined under (a).

Claim 1 requires that the nucleic acid encodes a complete or partial insect acetylcholine receptor subunit having the ability to form homooligomeric acetylcholine receptors when expressed in host cells.

Anticipation requires that every element of the claimed invention be disclosed in the prior art reference. *Akzo N.V. v. The United States International Trade Commission*, 808 F.2d 1471, 1479 (Fed. Cir. 1986), cert. denied, 482 U.S. 909 (1987). Celniker et al. teach the sequence of an alcohol dehydrogenase region of Drosophila melanogaster DNA, Liao et al. teach the sequence of a zebrafish stem cell leukemia protein (tal-1) mRNA and teaches that a SCL/tal-1 transcription factor acts downstream of cloche to specifically hematopoietic and vascular progenitors, while Vogel et al. teach the sequence of a Beta vulgaris (beet) cDNA for elongation factor 2. None of the cited references disclose a nucleic acid which encodes a complete or partial insect acetylcholine receptor subunit having the ability to form homooligomeric acetylcholine receptors when expressed in host cells, thus none of the references disclose every element of Claim 1.

Further, the Examiner alleges that Celniker et al. teach a sequence which is 99.4% identical to bases 9-836 of SEQ ID NO: 1 and which contains 340 bases identical to bases 496 to 836 of SEQ ID NO: 1. In contrast, Claim 1 recites a sequence according to nucleotide No. 372 to nucleotide No. 2681 of SEQ ID NO: 1, and sequences hybridizing to that sequence, complementary to that sequence, and encoding the same amino acid as that sequence. The 828 and 340 base sequences of Celniker et al. do not teach or suggest the 2310 base pair sequence of SEQ ID NO: 1 recited in Claim 1.

The Examiner alleges that Liao et al. teach a sequence which is identical to SEQ ID NO: 3 over a length of 79 bases. In contrast, Claim 1 recites a sequence according to nucleotide No. 335 to nucleotide No. 1822 of SEQ ID NO: 3, and sequences hybridizing to that sequence, complementary to that sequence, and

encoding the same amino acid as that sequence. The 79 base sequence of Liao et al. does not teach or suggest the 1487 base pair sequence of SEQ ID NO: 3 recited in Claim 1.

Vogel et al. teach GenBank Accession No. Z97178 which is identical to SEQ ID NO: 5 over a length of 98 bases. In contrast, Claim 1 recites a sequence according to nucleotide No. 95 to nucleotide No. 1597 of SEQ ID NO: 5, and sequences hybridizing to that sequence, complementary to that sequence, and encoding the same amino acid as that sequence. The 98 base sequence of Vogel et al. does not teach or suggest the 1502 base pair sequence of SEQ ID NO: 3 recited in Claim 1.

For the reasons set forth above, Claim 1 is not anticipated by the cited references, whereby the rejection of Claim 1 under 35 U.S.C. § 102 based on Celniker et al., Liao et al., or Vogel.

Claim 2-7 and 24-31 have been rejected under 35 U.S.C. § 103 as being unpatentable over any one of Schulte et al., Celniker et al., Liao et al., or Vogel et al. in view of Ausubel et al. The Examiner relies on Schulte et al., Celniker et al., Liao et al., and Vogel et al. as teaching the sequences discussed above. The Examiner alleges that Ausubel et al. teaches the introduction of nucleic acids into host cells and the use of vectors to do so.

As discussed above, Schulte et al. is not a proper reference, whereby the rejection based on Schulte et al. in view of Ausubel et al. is overcome.

As will be set forth below, Applicants submit that Claim 2-7 and 24-31 are not rendered obvious by any one of Celniker et al., Liao et al., or Vogel et al. in view of Ausubel et al. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

The deficiencies of the primary references with respect to Claim 1, on which Claims 2-7 and 24-31 depend, have been set forth above. Ausubel et al. broadly disclose methods of introduction of DNA into mammalian cells, such as calcium phosphate transfection, DEAE-dextran transfection, electroporation, and lipsome-mediated transfection. The addition of Ausubel et al. will not overcome the deficiencies of the primary references.

As discussed above, the 828 and 340 base sequences of Celniker et al. do not teach or suggest the 2310 base pair sequence of SEQ ID NO: 1 recited in Claim 1, the

79 base sequence of Liao et al. does not teach or suggest the 1487 base pair sequence of SEQ ID NO: 3 recited in Claim 1, and the 98 base sequence of Vogel et al. does not teach or suggest the 1502 base pair sequence of SEQ ID NO: 3 recited in Claim 1. Applicants find no teaching or suggestion in Ausubel et al. of the 2310 base pair sequence of SEQ ID NO: 1, the 1487 base pair sequence of SEQ ID NO: 3, the 1502 base pair sequence of SEQ ID NO: 3 recited in Claim 1, or of sequences hybridizing to those sequences, complementary to those sequences, or encoding the same amino acid as those sequences. Thus, none of the references, individually or combined, suggest the sequences recited in Claim 1.

Moreover, Applicants find no teaching or suggestion in the references, individually or combined, using the methods of Ausubel et al. in combination with a nucleic acid, comprising a sequence as recited in Claim 1. More particularly, Applicants find no teaching or suggestion in the references, individually or combined, of a vector which comprises at least one nucleic acid of Claim 1, as recited in Claim 2; a host cell which contains a nucleic acid of Claim 1, as recited in Claim 4; a nucleic acid of Claim 1(c) which comprises a sequence that hybridizes with a sequence defined under (a) in 0.5 x SSC at 60°C or in 0.2 x SSC at 60°C, as recited in Claims 22 and 23, respectively; or of a process for preparing a polypeptide encoded by a nucleic acid of Claim 1 comprising culturing a host cell containing a nucleic acid of Claim 1 or a vector comprising at least one nucleic acid of Claim 1 under conditions which ensure expression of the nucleic acid, and isolating the polypeptide from the cell or the culture medium, as recited in Claim 10.

Ausubel et al. may suggest it is obvious to try the methods taught by Ausubel et al. in order to introduce a nucleic acid into cells, however, obvious to try is not to be equated with obviousness under 35 U.S.C. §103. *Gillette Co. v. S. C. Johnson & Sons, Inc.*, 16 USPQ2d 1923, 1928 (Fed. Cir. 1990). The mere fact the prior art could be modified does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Gordon* 221 USPQ 1125, 1127 (Fed. Cir. 1984).

For the reasons set forth above, Schulte et al. is not a proper reference, and Claim 2-7 and 24-31 are not rendered obvious by any combination of Celniker et al., Liao et al., or Vogel et al. and Ausubel et al., whereby the rejection of Claim 2-7 and

24-31 under 35 U.S.C. § 103 based on any of Schulte, Celniker, Liao et al., or Vogel et al. in view of Ausubel et al. should be reversed.

For the reasons set forth above, Applicants submit that the claims herein are supported by an enabling description, definite, and neither anticipated nor rendered obvious by the cited references and combination of references. The Examiner is therefore requested to withdraw the rejection to the claims and to allow the application to pass to issue.

Attached is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version With Markings To Show Changes Made".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claims 8-9, 11-20, and 32-33 have been canceled.

Claims 1, 22 and 23 have been amended as follows:

1. (Twice Amended) An isolated nucleic acid [encoding a complete or partial insect acetylcholine receptor which comprises] comprising a sequence selected from

(a) a sequence according to nucleotide No. 372 to nucleotide No. 2681 of SEQ ID NO: 1, nucleotide No. 335 to nucleotide No. 1822 of SEQ ID NO: 3 [or] and nucleotide No. 95 to nucleotide No. 1597 of SEQ ID NO: 5,

(b) [a partial sequence at least 14 base pairs in length of the sequence defined under (a),

(c)] a sequence hybridizing with any of the sequences defined under (a) in 2 x SSC at 60°C,

[(d) a sequence exhibiting at least 70% identity with any of the sequences defined under (a) between position 1295 and position 2195 from SEQ ID NO: 1, or between position 432 and position 1318 from SEQ ID NO: 3, or between position 154 and position 1123 from SEQ ID NO: 5,

(e)] (c) a sequence complementary to the sequences defined under (a), and

[(f)] (d) a sequence which, due to degeneracy of the genetic code, encodes the same amino acid sequences as those encoded by the sequences defined under (a), [(b), (c) and (d)]

wherein said nucleic acid encodes a complete or partial insect acetylcholine receptor subunit having the ability to form homooligomeric acetylcholine receptors when expressed in host cells.

22. (Twice Amended) The nucleic acid of Claim 1(b) [(c)] which comprises a sequence that hybridizes with a sequence defined under (a) in 0.5 x SSC at 60°C.

23. (Twice Amended) The nucleic acid of Claim 1(b) [(c)] which comprises the sequence that hybridizes with a sequence defined in (a) in 0.2 x SSC at 60°C.

#21
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P.2.

PATENT APPLICATION
Mo5176
LeA 33,020

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION OF	
MARTIN ADAMCZEWSKI ET AL	GROUP NO.: 1632
SERIAL NUMBER: 09/303,232	EXAMINER: R. SCHNIZER
FILED: APRIL 30, 1999	
TITLE: NUCLEIC ACID WHICH ENCODE INSECT ACETYL-CHOLINE RECEPTOR SUBUNITS	

DECLARATION

1. I, Dr. Martin Adamczewski, declare that I am one of the co-inventors of the present U.S. Application No. 09/303,232, filed April 30, 1999, and I have reviewed and am familiar with the present Application including the claims; the Office Action dated November 8, 2000, issued therein and the prior art references relied on by the patent examiner in the Official Action.

2. I received my diploma degree in chemistry at the University of Freiburg, Germany in 1986. I entered the employ of Bayer, the assignee of the present application in 1993, where I have specialized in assay development. The following tests have been carried out under my supervision and direction.

3. HEK-293 cells which are untransfected do not exhibit a change in fluorescence after exposure to nicotine.

4. The data set forth in the specification in Figure 1 were obtained using HEK-293 cells transfected with a construct comprising the Hva7-1/5HT3 chimera described on page 14 of the specification, a nucleic acid in accordance with the invention. The cells were not transfected with any other constructs.

5. The data set forth in Figures 2-4 below were obtained by first isolating the Da7, va7-1 and Hva7-2 clones as set forth on pages 11-12 of the specification and preparing the constructs as set forth on pages 13-14 of the specification. In each experiment the cell culture, gene transfer and calcium concentration measurements (Fura-2 measurements) were performed as set forth on pages 14-15 of the specification.

6. The data set forth below in Figure 2 were obtained using HEK-293 cells transfected with a construct comprising the Da7 clone described on page 11 of the specification, a nucleic acid in accordance with the invention. The cells were not transfected with any other constructs.

7. The data set forth below in Figure 3 were obtained using HEK-293 cells transfected with a construct comprising the Hva7-1 clone described on page 13 of the specification, a nucleic acid in accordance with the invention. The cells were not transfected with any other constructs.

8. The data set forth below in Figure 4 were obtained using HEK-293 cells transfected with a construct comprising the Hva7-2 clone described on page 14 of the specification, a nucleic acid in accordance with the invention. The cells were not transfected with any other constructs.

Figure _

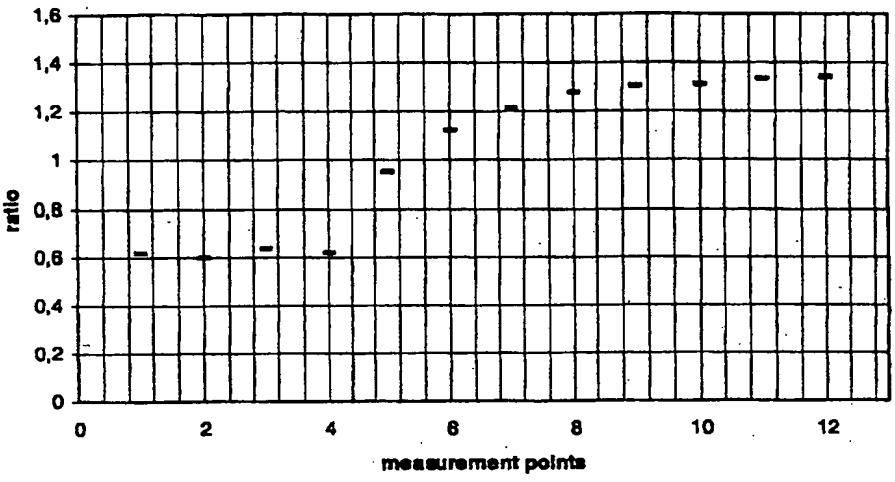


Figure 3

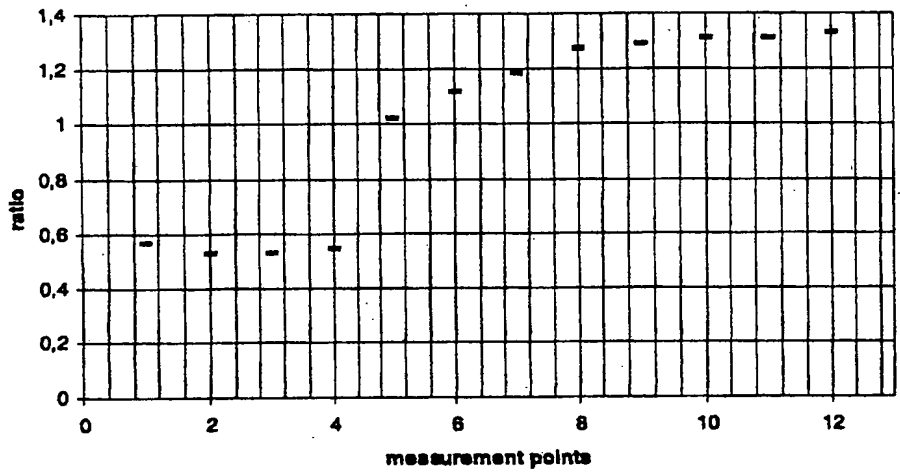
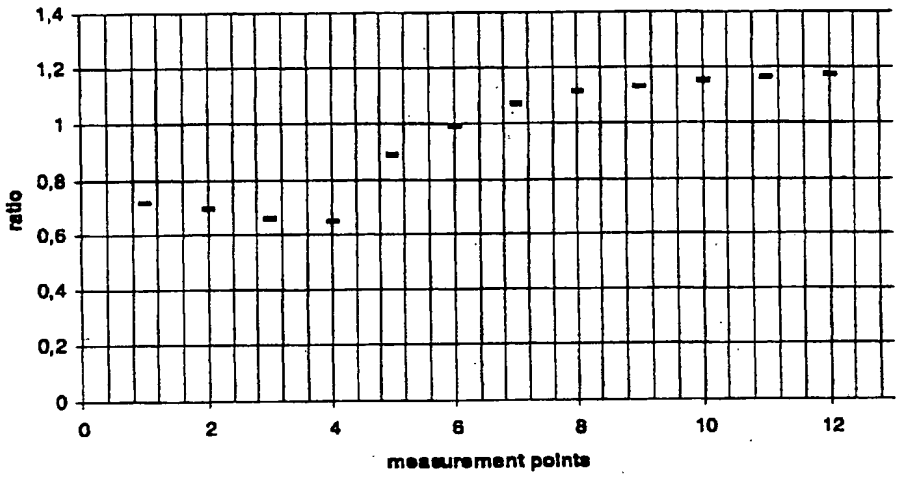


Figure 4



9. Figures 2-4 demonstrate that after transfection with constructs comprising a nucleic acid in accordance with the invention the transformed HEK-293 cells responded to nicotine by exhibiting an increase in calcium concentration.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true; and further that these statements were made with knowledged that willful statements and the like so made are punishable by fine or imprisonment, or both under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Dr. Martin Adamczewski

Date May 28, 2001

/jme/JAZ0086